Metabolic Effect of chronic lead administration and restraint stress on female Wistar rats.

ABSTRACT

Lead is a common environmental toxicant while restraint stress is primarily a psychological stressor. Lead exposure and psychological stressors, are known to exert adverse effects on metabolic health independently. The aim of this study is to investigate the metabolic effects of chronic lead acetate administration and restraint stress in female Wistar rats. Twenty-four (24) female Wistar rats weighing 180 - 240 grams were randomly divided into four (4) groups (n=6): Control (C), Restraint stress alone (RSA), Lead acetate alone (LDA), Restraint stress + Lead acetate (RSL). The duration of the study was 21 days. The lead acetate alone group were orally administered 100mg/kg of lead acetate, the restraint stress alone group were restrained for 1 hour daily and the restraint stress + lead group were administered lead acetate and restrained for 1 hour daily. Twenty-four hours post last lead administration and restraint conduction, all animal were anesthetized and sacrificed. Blood was collected via cardiac puncture for biochemical analysis. Results showed serum low-density lipoprotein (LDL), triglycerides (TAG) and total cholesterol (CHO) levels in rats exposed to lead alone and restraint stress alone groups were significantly increased ($p<0.05$) than those in control groups. The restraint + lead groups shows a significant increase in LDL, TAG and CHO levels when compared to the control, lead alone and restraint alone groups.Serum HDL levels shows no significant difference when compared to the control, restrain stress alone and lead alone groups. Serum glucose, insulin and cortisol levels in rats exposed to lead alone and restraint stress alone were significantly increased ($p<0.05$) than control groups. The restraint + lead groups showed a significant increase ($p<0.05$) in serum glucose, insulin and cortisol levels when compared to the control, lead alone and restraint alone groups. In conclusion, this study showed that exposure to restraint stress and lead has a significant effect on the metabolic profile of female Wistar rats.

KEYWORDS: ***Lead acetate, Restraint stress, Glucose, Insulin, Cortisol, CHO, LDL, HDL, TAG.***

1. Introduction

Heavy metals are a group of naturally occurring metallic elements characterized by their high density and potential toxicity at even low concentrations (Wu *et al.,* 2016). These elements including lead, mercury, cadmium, arsenic, and others, can be found in the Earth's crust but are also released into the environment through human activities, such as industrial processes and pollution (Rahman and Singh, 2019).Lead acetate, also known as lead diacetate, is a toxic heavy metal that accumulates in the human body and can cause severe health problems such as increased risk of high blood pressure, cardiovascular disease, and kidney damage. Additionally, studies have linked lead exposure to impaired glucose homeostasis, insulin resistance, visceral adiposity and increase endoplasmic reticulum stress (Martins et al., 2023).

Stress is a complex physiological and psychological response to external and internal environmental stimuli (Yang et al., 2024). Restraint stress is a widely used experimental paradigm in behaviouralneuropsychatric disorders (Italia et al., 2020). This method involves the immobilization of animals, typically rodents in a confined space, which induces both physical and psychological stress responses (Shoji and Miyakawa, 2020). Research has shown that such stressors can lead to significant changes in behavior, neuroendocrine function, and brain morphology, making them valuable for studying the mechanisms underlying stress-related conditions.

Metabolic profile is a comprehensive analysis of an organism’s metabolic state, which involves measuring a wide range of endogenous and exogenous molecules (Yu et al., 2012). Metabolic profile includes insulin resistance, pancreatic beta cell function, glycated hemoglobin and lipid profile (Olugbemidebe et al., 2022). Exposure to stress, both acute and chronic stress, are involved in metabolic dysfunction (Bouillon-Minois and Dutheil, 2022). However, studies involving the combined influence of restraint stress and lead acetate on metabolic profile of female Wistar rats is limited. Therefore, this study

seeks to evaluate the effect of restraint stress and lead acetate administration on the metabolic profile of female Wistar rats.

2.0 Material and Methods

2.1 Chemicals and Compounds

Lead acetate (Kermel, China), Chloroform, Normal saline, distilled water, Formosaline, phosphate buffer saline was purchased from Science laboratory, LAUTECH, Oyo state, Nigeria.

2.2 Study Design

Twenty-four (24) healthy adult female Wistar rats weighing 180-240g were purchased from the Department of Physiology, Animal Laboratory (Oyo state, Nigeria) and kept under a standardized laboratory environment (12/12 h light/dark cycle). The rats were acclimatized for two weeks and were allowed free access to animal feed and water *ad libitum.* All animals received humane care in compliance with the Guidelines of the Animal Research Ethical committee of Ladoke Akintola University of Technology. This animal experiment was approved by the Institutional Animal Research Ethical Committee (APPROVAL NO: ERCFBMSLAUTECH:059/08/2024).

After acclimatization, the rats were randomly divided into four groups with six (6) rats in each group. Group I represent the control group while groups II, III, IV served as the experimental groups. The group designate are: I=Control group (CTL), II=Restraint Stress Alone (RSA), III=Lead Alone (LDA) and IV= Restraint Stress + Lead (RSL). The table below shows animal grouping and summary of experimental procedure:

Table1: Animal grouping and experimental procedures

GROUPS	ADMINISTRATION
Control (CTL)	Rats were given only animal feed and water *ad libitum* for 21 days.

Restraint stress alone (RSA)	Rats were subjected to restraint stress using wire gauze for 1 hour daily for 21 days.
Lead alone (LDA)	Rats were administered lead acetate (100 mg/kg) orally for 21 days.
Restraint stress + Lead (RSL)	Rats were administered lead acetate (100 mg/kg) daily orally and were subjected to restraint stress using wire gauze for 1 hour for 21 days.

2.3 Sample preparation

Twenty-four hours after the last lead acetate administration and restraint stress conduction, the animals were anesthetized by placing them in desiccator with a chloroform soaked cotton wool. Blood samples was collected via the cardiac puncture into sample bottles. Serum was obtained from the collected blood by centrifuging at 2500 revolutions for 10 minutes. The obtained serum were stored at -80□ until use.

2.4 Biochemical Assays

High-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol (CHO) and triglycerides (TG) were assayed using commercial kits and standardized methods. Serum Insulin and Cortisol were assayed using ELISA kits. Serum glucose was assayed using a glucometer.

2.4.3 STASTICAL ANALYSIS

All results obtained are expressed as Mean ± Standard Error of the Mean (S.E.M). Statistical analysis of the glucose, insulin and cortisol results were performed using GraphPad Prism (version 5.0). Each mean value was compared by one way analysis of variance (ANOVA) and statistical differences between groups using Tukey's *posthoc*test. Lipid profile results were performed using SPSS (version 16.0) with Duncan *posthoc*. $P<0.05$ is considered significant.

3. Results and Discussion

Results

Table 2: Effect of restraint stress and lead acetate administration on lipid profile in female Wistar rats.

	CTL	RSA	LDA	RSL
CHO (mg/dl)	108.77±5.22[a]	170.12±4.47[b]	167.85±4.45[b]	189.12±4.56[c]
LDL (mg/dl)	42.33±2.42[a]	60.42±2.56[b]	84.68±3.37[c]	90.56±5.22[d]
HDL (mg/dl)	35.52±2.33[a]	38.18±3.62[a]	31.82±2.2[a]	39.22±2.56[a]
TAG (mg/dl)	56.01±2.56[a]	75.11±3.65[b]	90.65±4.41[c]	91.56±4.56[c]

Serum CHO, LDL, TAG levels in rats exposed to RSA, LDA and RSL were significantly increased ($p<0.05$) than those in control groups. The restraint + lead groups however showed a significant increase in CHO and LDL levels when compared to the control, lead alone and restraint alone groups.Serum HDL levels shows no significant difference when compared to the control, restrain stress alone and lead alone groups.

Values are expressed as mean ± SEM (n=6). Groups with superscripts of different letters are significantly (p<0.05) different from each other. Groups with superscripts of same letters are not significantly (p<0.05) different from each other.

FigureShowing the effect of restraint stress and lead acetate administration on metabolic parameters in female Wistar rats.

A **B**

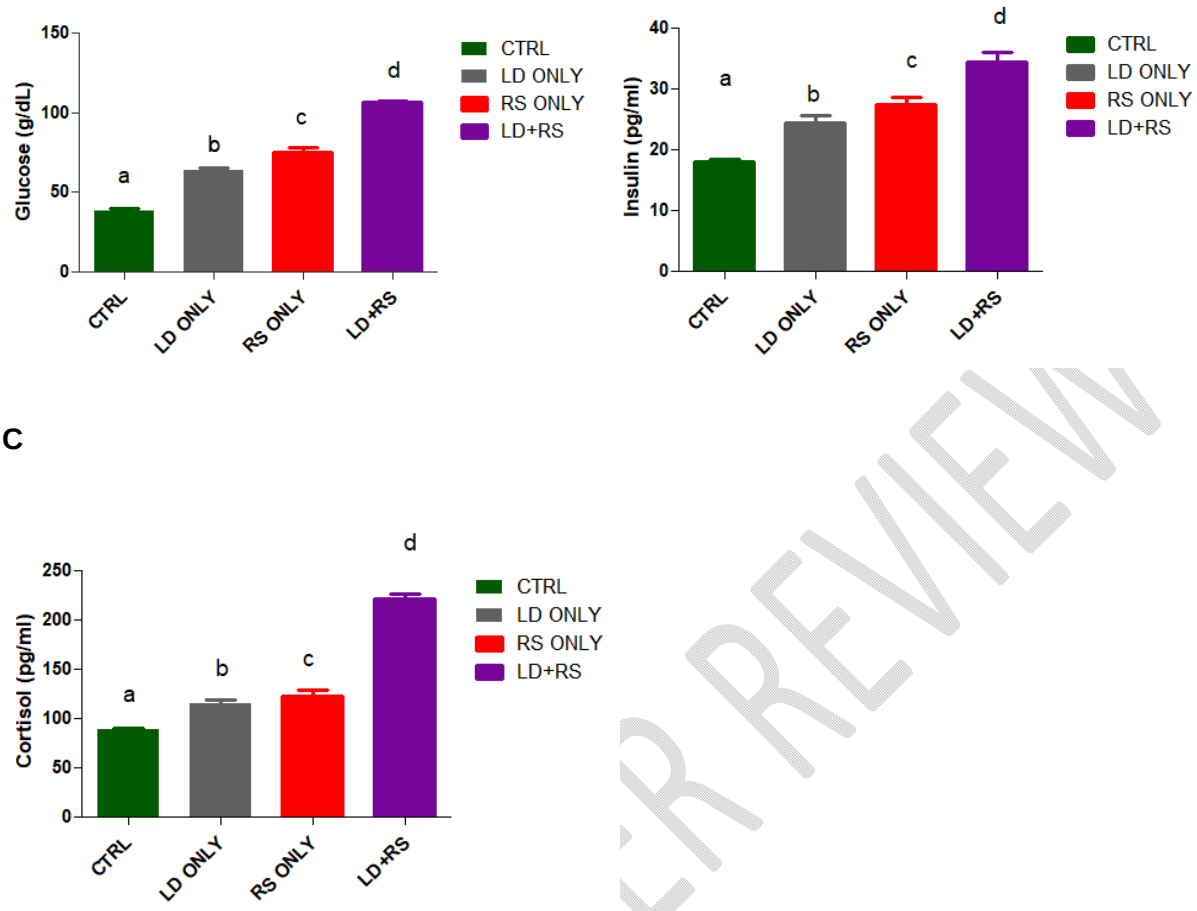

Figure 1

Effect of Restraint stress and Lead acetate administration on serum glucose (A), insulin (B) and cortisol (C) in female Wistar rats.

Values are expressed as mean ± SEM (n=6). Bars with superscripts of different letters are significantly (p<0.05) different from each other. Bars with superscripts of same letters are not significantly (p<0.05) different from each other.

DISCUSSION

Result observed in the lead alone group is consistent with previous research of Alya et al., 2015 where cholesterol (CHO), low-density lipoprotein (LDL) and triglycerides (TAG) concentration were significantly

increased following lead intoxication in all treated groups compared to their relative controls. Lead accelerates lipid peroxidation and degradation of polyunsaturated membrane lipids and lipoproteins resulting in alteration of lipid metabolism (Chlubek and Baranowska-Bosiacka, 2024). Increased cholesterol levels may be due to lead-induced activation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and HMG CoA synthase (Sawada et al., 2005) or due to inhibition of cytochrome P-450 resulting in the limitation of bile biosynthesis which is the only significant route for the elimination of cholesterol (Alya et al., 2015). Result observed in the restraint group compared to control is consistent with the previous study of Ahn et al., 2016 where restraint stress induction increases LDL-cholesterol, triglycerides and total cholesterol levels by 150-170%. The result of this current study showed that CHO, LDL and TAG levels was significantly increased in restraint + lead groups. This result indicates that the combination of restraint stress and lead greatly induces alteration of lipid metabolism.

High density lipoprotein (HDL) levels showed no statistical significance across all groups. HDL levels are tightly regulated by the body to maintain lipid homeostasis. Despite external stressors or exposures, the body may prioritize maintaining HDL levels within a certain range (Duan et al., 2022). This study might have been conducted over a relatively short period, during which changes in HDL levels might not have been significant enough to detect differences between groups. Other mechanisms or compensatory responses within the body might be at play thus, mitigating the effects of lead exposure or stress on HDL levels.

Serum glucose, insulin and cortisol was significantly increased in the restraint alone and lead alone group compared to control groups. Results observed in the restraint alone group is in correlation with previous studies where fasting serum glucose concentration, insulin and cortisol concentration were elevated in restraint group compared to control indicating that restraint stress exacerbated glucose intolerance and insulin resistance in rats (Matsuura et al., 2015; Morakinyo et al., 2016). Increased glucose levels may be related to the enhanced activity of the hypothalamic-pituitary adrenal axis during stress resulting in increased secretion of adrenocorticotropic hormone and corticosteroids into the circulation. The release of ACTH increases cathecolamine production from the adrenal to mobilize carbohydrate reserves from the tissues resulting to elevated blood glucose levels (Morakinyo et al., 2016). Insulin resistance can be due

to stress-induced corticosteroid secretion. This is consistent with similar reports in which insulin resistance can be induced by cortisol administration in rodents (van Donkelaar et al., 2014).

Results observed in the lead alone correlates with previous studies in which both fasting glucose and insulin levels was impaired in rats exposed to lead. Impairments of glucose tolerance may be as a result of defects in insulin secretion and/or insulin sensitivity. In correlation to previous studies, lead was significantly associated with higher ACTH:CORT ratios, which is consistent with an decreased adrenal response to endogenous ACTH. Exposure to lead can impair negative feedback loop of the hypothalamic-pituitary adrenal axis leading to higher adrenocorticotropin release during stress response (Fortin et al., 2012). Serum glucose, insulin and cortisol levels was significantly increased in restraint + lead groups compared to control, restraint alone and lead alone groups. The current result might have resulted from the combined mechanisms of lead-induced alteration of the hypothalamic-pituitary adrenal axis, glucose intolerance and insulin insensitivity caused by restraint stress resulting in metabolic dysfunction.

4. CONCLUSION

In conclusion, this present study have shown that the combined exposure to lead and chronic stressors exacerbated the dysregulation of lipid metabolism and glucose homeostasis.

Institutional Review Board Statement

This study was conducted following the guidelines set by the Animal Ethical Committee of the Faculty of Medical sciences, Ladoke Akintola University of Technology, Oyo, Nigerial and the regulations were adhered to throughout the research process.

Informed Consent Statement

Not applicable.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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